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(54) Title: USE OF SYNTHETIC PEPTIDES TO GENERATE AND MANIPULATE CELLULAR IMMUNITY

(57) Abstract

Methods and compositions are provided for treating host prophylactically or therapeutically to stimulate the CTL of the host against the disease state. Particularly, a binding domain is provided which binds to one or more MHC Class I antigens, changing the restriction of the Class I antigen, so as to provide for an allogeneic response. By joining the binding domain or α helix to an epitope of an antigen of interest, the host may be stimulated to mount a CTL response in case of subsequent exposure or an existing disease state.

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USE OF SYNTHETIC PEPTIDES TO GENERATE AND MANIPULATE CELLULAR IMMUNITY

INTRODUCTION

Technical Field

The field concerns the manipulation of the immune system relating to Class I antigens.

Background

The Class I transplantation antigens, H-2D, K, and L in the mouse and HLA-A, B, and C in the human, encoded within the major histocompatibility complex are polymorphic cell surface glycoproteins which play a central role in the regulation and function of the immune system. These molecules act as antigen presentation structures or "restriction elements" directing the attack of cytotoxic T-lymphocytes (CTL) against virally infected or non-self target cells. CTL generally show a requirement for the co-recognition of foreign antigens in the context of specific self MHC Class I products. Therefore, CTL recognition is said to be "MHC-restricted."

The immune system is a major determinant of the health and welfare of the mammalian host. Despite the extraordinary panoply of mechanisms of the immune system, mammalian hosts still remain susceptible to a wide variety of diseases as a result of infection by microorganism and viral pathogens and of the occurrence of aberrant cells, such as neo-plastic cells. While for the most part, the mammalian host is able to overcome most diseases, not infrequently a significant period of debilitating illness with varied consequences to the mammalian host may result. For the immune sys-

tem the protection afford d is related to the various mechanisms of invasion and regulation of the pathogen, to the nature of the diseased cell, and to the particular susceptibility of various individuals to diseases.

There is substantial interest in being able to manipulate the immune system to enhance its ability to overcome disease states by attacking and eliminating pathological or diseased host cells.

Relevant Literature

Townsend et al., Cell (1986) 44:959-968 and Townsend, Nature (1986) 324:575-577 suggest that certain virus-specific CTL recognize virally-derived processed peptides in association with self-Class I. Kourilsky et al., Proc. Natl. Acad. Sci. USA (1987) 84:3400-3404 report investigation of the interaction between allospecific T-cells and the allo-MHC molecule. Recent studies include Maryansky et al., J. Immunol. (1986) 136:4340-4347 and Maryansky et al., Nature (1986) 324:578-579. Clayberger et al., Nature (1987) 33:763-765 describe the effect of A2 peptides on cytolysis.

SUMMARY OF THE INVENTION

Methods and compositions are provided for modulating the immune response of a host to a foreign antigen by providing a peptide comprising at least one of the helices of the polymorphic region of a foreign Class I antigen. The compositions may be employed to enhance the immune response in relation to a foreign MHC or other antigen or to tolerize the host to a foreign MHC.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for modulating the immune system in relation to Class I restricted elements. Peptides which bind to the native MHC providing an epitope of interest may be employed. By binding of the peptide to the native MHC, one can provide for enhancement of immune response to an antigen of interest or tolerization toward a particular epitope.

The subject peptides are characterized by having at least a partially non-contiguous sequence which is primarily hydrophobic and binds to the β-pleated sheet of a designated host Class I major histocompatibility antigen (MHC). The sequence may be the same as or substantially similar to the β-sheet binding sequence of an α-helix of the designated Class I antigen itself. The intervening amino acids between the binding sequence may be the sequence of an α-helix of a Class I antigen other than the designated Class I antigen or the same sequence. Where different, the sequence will be chosen to stimulate or tolerize T-cells to an antigen of interest.

The compositions are characterized by having a sequence of at least 10 amino acids which are naturally in an α-helix conformation or may be induced into such conformation by binding to a β-sheet of the cleft of a Class I MHC. The sequence will be further characterized by having hydrophobic residues spaced from 2 to 3 amino acids apart, where the hydrophobic residues are conserved, (that is, are the same amino acid or may be substituted by a conservative substitution), which sequence is referred to as the agretope. Conservative substitution is intended such that the substituted amino acid differs by no more than 3, (usually by no more than 2 carbon or other heteroatoms, particularly

chalcogen), atoms and th re will be no significant difference in polarity, (that is charged amino acids and the highly polar amino acids, such as asparagine or glutamine will usually not be employed).

The amino acids may be divided into groups which are non-polar and polar, and further divided into low and high polarity, where the low polarity amino acids may be readily substituted for the non-polar amino acids. The non-polar aliphatic amino acids are: G, A, P, V, L, I; the non-polar aromatic amino acids are: F, W, Y; the low polarity amino acids are: H, C, M, S, T; and the high polarity amino acids are: D, E, K, R, N, Q.

The amino acid sequence associated with and binding to the β-sheet will be referred to as the binding domain. The binding domain will be comprised of two parts, the amino acid residues associated with binding to the β-sheet and occupying a region of the MHC cleft ("binding sequence") and the amino acid residues interspersed between the binding residues ("epitope"), where amino acids of one group may participate to varying degrees in the handling of the other group to a complementary region. The individual interspersed residues will be referred to as the epitopic residues, which in varying degrees participate in and define the epitope of the binding domain. These residues will be varied widely depending upon the function and purpose of the binding domain. The epitopic residues will be involved in the recognition by the T-cell receptor of the MHC antigen to which the binding domain is bound.

For the most part the epitope will include at least 1, usually 2, hydrophilic residues, frequently charged. By using a pattern of alternations of the relevant portion of the Class I antigen, where the alt rnations provide for a hydrophilic sequence with an intervening primarily hydrophobic sequence, the amino

acids involved in defining the epitope may be identified. These may be substituted with amino acids associated with an epitopic site of an antigen of interest. In this way novel peptides are designed to provide a T-cell response to a particular epitopic site.

The peptide which is employed will have at least 10 amino acids, usually at least about 12 amino acids, preferably at least 14 amino acids. It may have 200 amino acids or more, usually having not more than about 60 amino acids, more usually having not more than about 36 amino acids. The binding domain will usually occupy at least 10, usually 14, amino acids, will usually be not more than about 20 amino acids, more usually not more than about 18 amino acids, among these, at least 3 being binding residues. The remaining amino acids of the peptide may be present for a variety of reasons. Included among the remaining amino acids, particularly those amino acids contiguous with the binding domain, may be amino acids providing for a second epitope common to an antigen of interest which may be involved in T-cell stimulation. Alternatively or in addition, the additional sequence or extended sequence may provide for stability of the peptide, direction of the peptide to a particular cellular compartment, ease of administration, or the like.

For the most part, peptides of fewer than about 60 amino acids will be preferred, since these can be synthesized employing automated synthesizers. However, peptides of about 35 or more amino acids, can be obtained employing recombinant DNA technology, by devising DNA sequences encoding the desired peptide, inserting the sequence into an expression vector. These vectors are now generally available or can be readily made, and can be transformed into an appropriate host for expression and isolation of the desired peptide.

In devising peptides different from the natural sequence of the Class I antigen, a pattern is defined in a helix domain, either the α-1 or α-2 helix domain. A turn of the helix is something greater than 3 amino acids, approximately 3.2-3.6 amino acids, and one can define those amino acids which will likely be on the upper portion or top of the helix and those which will likely be at the lower portion or bottom of the helix. The lower amino acids will be involved in non-covalent binding to the β-sheet, whereas the upper amino acids will be involved in defining the epitope and binding to the T-cell receptor, in varying degrees. The epitopic sequence will be primarily a hydrophilic sequence, while the agretopic sequence will primarily be hydrophobic.

For the most part, there will be a pattern of (a b b a a b b a a), where "a" intends a hydrophilic amino acid and "b" a hydrophobic amino acid. This pattern will have to be corrected for the fact that a turn is not exactly 3, so that amino acids will have to be inserted to correct for the extra amino acids. To be more exact, the amino acids should be indicated on a circle as to their relative positions in space and those amino acids which provide a hydrophilic surface may be considered the upper amino acids involved in the T-cell recognition.

If one wishes to modify the sequence to change the epitope, so as to change the subset of T-cells that are stimulated by the peptide, one may retain the amino acids defining the agretope and relate the remaining amino acids to an epitope of interest. The amino acid sequence would be treated in the same manner as the Class I sequence to define those amino acids involved in the topography of the epitope of interest. In defining the amino acids for the epitope, usually the sequence employed would be a T-cell stimulating sequence of the antigen as the epitope of interest.

The T-cell stimulating sequence of the antigen may be one which is restricted by the haplotype of the T-cells to be stimulated. One would use the circle map of the sequence to define those amino acids which are spatially related and provide a surface to which a T-cell receptor might bind.

The hydrophobic sequence may be common to a number of different Class I antigens although this will have to be verified as to each haplotype. That is, one would normally select a binding sequence similar to or the same as the binding sequence of a helix of the haplotype of the host. Otherwise, one would use the particular sequence of the α-helix of the haplotype of the host cells. Since the host will normally be heterozygous for each Class I antigen, one could choose to stimulate only one subset of T-cells restricted by one haplotype or have two or more α-helices for the different haplotypes. In some instances, one helix could be chosen over another due to greater or lesser homology between the host Class I α-helix and the α-helix of the foreign antigenic sequence.

Illustrative of the methodology is the following example. The Class I antigen employed is H-2L^d, while the epitope of interest will be an epitope of influenza virus. The following is the sequence of the α-1 domain of the H-2L^d Class I antigen.

61 2 3 4 5 6 7 8 9 70 1 2 3 4 5 6 7 8 9 80 1 2 3 4 5
E R I T Q I A K G Q E Q W F R V N L R T L L G Y Y

where the underlined letters are those which are likely to be involved with the epitope.

As exemplary of an epitope of interest will be a sequence of the influenza virus.

I A S N E N M D A M E S S T L E
I A S N E NM D A M E S S T L E
I A S N E N M D A M E S S T L E
I A S N E N M D A M E S S T L E

By substituting the amino acids involved with the epitope in 4 different orientations to allow for the fact of the 3 plus a fraction of amino acids involved with a turn, one prepares 4 different hybrid peptides comprising the agretope of the Class I antigen and the epitope of the influenza virus. Thus the 4 hybrids would be:

E R I I A I A E G Q D A W F S V N L E T L L G Y Y
E R I T I I A N G Q M D W F E V N L L T L L G Y Y
E R I T Q I A S G Q N M W F M V N L T T E L G Y Y
E R I T Q I A A G Q E N W F A V N L S T L E G Y Y

By employing the hybrids in conjunction with cells of an allogeneic host and T-cells restricted by the cells of the allogeneic host, one would stimulate a subset of T-cells recognizing the epitope displayed by the hybrid peptide. At least one of the stimulated T-cell subsets would be active toward a cell infected with influenza virus.

The peptide need not be limited to a single modified sequence, but a string of such sequences may be joined together, either directly or through linking or spacing sequences, usually short sequences of no more than about 30 amino acids. With viruses or other microorganisms, one may encounter a large number of sequences which would be presented to T-cells. Each of the epitopes of these sequences could be substituted for the epitope of the Class I α-helix sequence.

The subject peptides could be used in the case of autoimmunity. Autoimmunity may be associated with cross-reactivity betwe n an antigen to which the host

has mounted an immune response ("pathogenic antigen") and a native surface antigen, e.g. a transplantation antigen. In most, if not all, cases, the epitope of the pathogenic antigen will not be identical to the epitope of the native antigen. By modifying the Class I sequence to result in an epitope more similar to the pathogenic antigen than the epitope of the native antigen, the peptide may be used as a vaccine. One stimulates the immune response to mount a more effective response to the pathogenic antigen to result in stimulated T-cells which are less stimulated by the native antigen than the pathogenic antigen. The change in response would serve to protect a host from an autoimmune disease to which the host might be susceptible, due to cross-reactivity between a native antigen and a foreign antigen.

There are many situations where one wishes to elicit an immune response. These situations may include various pathogenic diseases as a result of invasions by microorganisms, such as bacteria and viruses. Cellular microorganisms may include mycoplasma, or viruses including DNA or RNA viruses, such as retroviruses, rhinoviruses, picornaviruses, FeLV, influenza virus, visna viruses, paramyxoviruses orthomyxoviruses, hepatitus virus, papovavirus, reovirus, parvovirus, herpesvirus, rhabdovirus, etc.

The binding domain of the Class I antigen may be bound to additional peptide sequences, particularly immunodominant sequences, normally foreign, for a variety of purposes. Particularly, the Class I binding domain may be bound to one or more immunodominant sequences which are restricted by the Class I haplotype of the host. This will have the effect of greatly enhancing the binding of the foreign domain to the Class I antigen providing for a stronger immune response. In addition, if the foreign antigen and the epitope of the binding domain are associated with th

same entity, two different subsets of T-cells will be stimulat d. Thus, when either of the epitopes are presented upon infection, there will be enhanced populations of previously stimulated T-cells.

The domains of foreign antigens presented by Class I antigens may be known for specific entities, e.g. viruses or other pathogens, or may be readily determined. These domains will fit the constraints described above for the Class I α-helix. Namely, based on the helical conformation, there will be a hydrophobic face and a hydrophilic face. Also, there may be some conservation between the hydrophobic face of a helix of the Class I antigen and the hydrophobic face of the domain of the foreign antigen. In any event, where the sequence is not known, there will be relatively few partial sequence candidates among the antigen sequence to be tested for T-cell response.

Various techniques can be employed to simplify the selection of the candidates for the immunodominant sequence. The immunodominant sequence refers to a sequence of an antigen which binds to the Class I antigen haplotype of interest. For example, there is the Rothbard algorithm, which requires a sequence in either the N-C or C-N direction of the first amino acid being glycine or charged, the next two amino acids, being non-charged, usually non- or low-polarity, and one of the next two amino acids being polar. Sequences including this algorithm are likely candidates for an immunodominant sequences. In addition, where immunodominant sequences of antigens restricted by the same haplotype are known, these sequences may be compared to the antigen of interest for homology as to the binding residues and their spacing, to further simplify the selection of the immunodominant sequence.

The binding domain and additional peptide sequence(s) need not b joined directly together, but may be bridged with up to about 30 amino acids to space

the sequences apart. The particular number of amino acids is not critical and will usually be primarily one of convenience. Where more than one foreign sequence is involved, the foreign sequences may be associated with the same entity, e.g. antigen of the same virus, or with different entities, e.g. antigens of different viruses. Usually, there will be fewer than about 10 different foreign sequences, and usually the same sequence will not be repeated, although there may be some interest in having the same sequence repeated.

Alternatively, one may wish to tolerize the host against a particular major histocompatibility complex antigen (MHC). In this situation, one would normally use an identical sequence to one or both of the α-helices of the MHC's of the transplanted organ. By providing an injection of a high level of a peptide comprising one or both of the α-helices, one could tolerize the host to the novel MHC, thus reducing the immune response.

The subject compositions may also be employed with those hosts who have an MHC allele which does not respond to a particular pathogen. By employing a binding domain with an epitope cross-reactive with the particular pathogen, one could enhance the host's response upon infection with the antigen.

The subject compositions may also be used in case of neoplasia, where the neoplastic cells produce a surface protein which occurs either infrequently or at very low concentration on normal cells of the host. One could develop a T-cell population which recognized the naturally occurring protein in relation to the native MHC and would be effective in killing native neoplastic cells.

The subject compositions may be administered as a single peptide or a mixture of peptides, depending upon the purpose and function of th composition. Where the composition is directed to a particular

segment of the population which can be treated with the single binding domain, then a single compound may suffice. For example, a substantially universal binding domain or at least a binding domain which can bind to Class I MHC's of the major population groups would suffice joined to an antigenic epitope cross-reactive to a particular pathogen in providing for a T-cell immune response to the pathogen.

While the primary host of interest is the human, the subject invention may find use with any vertebrate, particularly domestic animals, pets, animals in zoos, or the like. In each case, the different binding sequences of the Class I antigens will be determined and employed.

The formulations will be affected by the manner in which the formulation is administered, the particular nature of the peptide, the intended lifetime of the peptide, or the like. The formulations may include lyophilized powders, dispersions in aqueous or alkanolic media, e.g. saline and phosphate buffered saline, in liposomes, as aerosols, etc. The concentration and amount administered may vary widely, depending upon the particular composition, the manner of administration, and the host response. Normally, at least about 0.01 μg/kg of host, more usually about 0.05 μg/kg of host, will be employed, and not more than about 10 μg/kg of host, more usually not more than about 5 μg/kg of host. The concentration will generally be from about 10 μg/ml to 1 mg/ml. The amount used will vary with the size of the host, the upper range being used with smaller hosts.

The manner of administration may be subcutaneous, intravascular, intraperitoneally, intramuscular, by inhalation, orally, or the like. One or more administrations may be made, and when used as a vaccine, particularly at at least two week intervals and up to

bi-annual intervals, although random repetitive administrations may be called for, particularly as a special need arises.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

MATERIALS AND METHODS

Generation of CTL

C3H mice were immunized by i.p. injection of 10^7 1591 cells expressing the A149 antigen as well as the A216 and A166 antigens (Linsk, et al., J. Exp. Med. (1986) 164:794-813). Spleens were harvested 4 weeks later and a secondary culture of 1591-tumor specific CTL was established by using mitomycin c treated 1591 tumor cells in complete medium (RPMI medium containing 10% FCS and 10^{-6}M 2-mercaptoethanol). After 1 week of growth, effector populations were transferred to L-cell stimulator cells transfected with either H-$2L^d$ or H-$2L^{149}$ to enrich for specific CTL populations. Cultures are maintained with L-cell transfectant stimulators or with L-cells in presence of 61-85 H-2L peptide (10µg/ml) in CTL medium (complete medium with 15% rat ConA supernatant).

Allospecific Cloned CTL

Allospecific CTL clone L3 was derived from a C57BL/6 anti-DBA/2 mixed lymphocyte culture (MLC) Glasebrook and Fitch, Nature (1979) 278:171-173 (received from Dr. O. Kanagawa,, Scripps Institute, La Jolla, CA). Allospecific CTL clones L9.4, L13.D4 and L.13.D.8 have been previously described (Linsk, et al., supra) and were received from Dr. J. Forman, University of Texas, Health Sciences Center, Dallas, TX. L9.4 was derived from a (C3Hxdm2)F1 anti-BALB/c culture while

L13.4 and L13.D8 was derived from a CBA anti-A.AL culture. All clones have been shown to be reactive to H-2L^d and are maintained in CTL medium with mitomycin treated BALB/c splenocytes as stimulators.

Target cell Lines and Animals

Murine tumor cell lines P815 (H-2^d), EL4(H-2^b) and C3H Ltk$^-$ cell (H-2^k) were used as target cells. K2a-7 and H23-12.1 are L-cell transfectants of H-2L^d or H-2L^{149} genes, respectively. The following mice are available from Jackson Laboratory (Bar Harbor, ME): C3H/HeJ(K^{k}D^k), BALB/cJ(K^{d}D^{d}L^d), BALB/c H-2^{dm2}(K^{d}D^d), BUB/BnJ(K^{q}D^q), A.CA/SnJ(K^{f}D^f), RIIIS/J(K^{r}D^r), ASW/SnJ(K^{s}D^s). B10.RKR.1(K^{k}D^r), and B10.ASR2(K^{k}D^s) mice were received from Dr. Chella David, Mayo Clinic, Rochester, Minnesota. Splenocytes were Concanavalin A induced for 48h and used as targets. A hamster line, tk$^-$ts 13 (ATCC CRL 1632), and the human B-lymphoblastoid line, WIL 2C 13 (Levy et al., Cancer (1968) 22:517-524) were also used as targets.

Synthesis of Peptides

Peptides were synthesized by using an Applied Biosystems model 430A automatic peptide synthesizer, then cleaved from resin and deprotected by using hydrogen fluoride (Penninsular Lab Inc., Belmont, CA and Applied Biosystems Inc., Foster City, CA). They were chromatographed on Sephadex G-10 using 10% acetic acid following lyophilization. The peptide was then purified by reversed phase HPLC at room temperature on a Brownlee 20 micron, 300Å, 25 x 1 cm Aquaphoroctyl Prep-10 cartridge column using 0.1% trifluoroacetic acid with a gradient of acetonitrile. Peptides were resuspended in 30% propanol at a concentration of 2mg/ml and further diluted to appropriate concentrations with assay medium.

^{51}Cr Release Assay

The ^{51}Cr labelled target cells were transferred to 96 well microtiter plate along with appropriate aliquots of peptides and brought to a total volume of 100µl with CTL medium. After 30 min. of incubation at room temperature, cells were washed 3X and resuspended in 100µl of CTL medium. CTL at various effector to target ratios were tested in a 4 hr assay. The specific percent ^{51}Cr released was determined as follows: (ER-SR)/(FR-SR) X 100, where ER is the average experimental CTL mediated release; SR is the average spontaneous release; and FR is the average full release from the target cells lysed in 150µl of RPMI containing 50µl of 1% NP-40.

RESULTS

Generation of H-2L Reactive CTL

1591 is a UV induced C3H fibrosarcoma which expresses several novel Class I epitopes not normally found on C3H tissue in addition to normal $H\text{-}2^k$ products (Phillips et al., Proc. Natl. Acad. Sci. USA (1985) 82:5140-5144). Previously, three novel Class I genes from the 1591 genome were cloned, whose products account for the expression of these epitopes and the recognition of these products by several CTL clones as well as viral-specific and alloreactive CTL cultures was examined (Linsk, et al., supra; Strauss, et al., J. Immunogenics (1986) 13:101-111). One of the novel genes, designated A149, is highly homologous to the $H\text{-}2L^d$ gene derived from BALB/c and encodes a distinct product which is recognized by a subset of $H\text{-}2L^d$ reactive CTL clones. The product of this gene is referred to as $H\text{-}2L^{149}$. The $H\text{-}2L^{149}$ molecule is identical to the $H\text{-}2L^d$ antigen in the $\alpha 1$ domain and differs by only 6 amino acid substitutions in the $\alpha 2$ domain. Functionally, this product can be distinguished from $H\text{-}2L^d$ by

the lack of reactivity with two H-2L^d-reactive mAb and by its inability to serve as a targ t for several other H-2L^d-specific CTL clones.

A distinct set of H-2L reactive CTL was generated, which are specific for the α1 domain of H-2L^d, by taking advantage of the identity between H-2L^d and H-2L^{149} in this region. Briefly, spleen cells from 1591-immunized C3H mice were cultured in vitro in the presence of mitomycin c treated 1591 tumor cells expressing H-2L^{149}. After one week of growth in vitro, cultures were stimulated with C3H L-cell transfectants expressing H-2L^d. Since CTL require specific antigenic stimulation in order to grow in the presence of interleukin-2 containing medium, only those cells reactive with the H-2L^d stimulator cells would be expected to grow out under these conditions. Consequently, stimulation of the anti-1591 CTL by H-2L^d transfectants enrich for CTL which preferentially recognize structural features shared between the H-2L^{149} and H-2L^d products, namely CTL epitopes encoded within the α1 domain. Indeed, CTL generated in this manner exhibited anti-H-2L^d specific reactivity, lysing both the H-2L^d and A149 transfectants, while showing no lysis of syngeneic L-cells.

Recognition of an H-2L Peptide by Cross Reactive CTL

Since the CTL were selected on the basis of the cross-reactivity between H-2L^d and H-2L^{149} products expressed on an H-2^k haplotype cells, these CTL are capable of recognizing a peptide from the region common to both H-2L molecules and as shown below in association with H-2^k restriction element. A peptide was synthesized corresponding to amino acids 61-85 form the α1 domain of H-2L^d. This peptide was chosen because it is derived from a r gion shared by the two H-2L molecules which is highly polymorphic among several other H-2 products. This region corresponds to the α helical

region predicted for Class I antigen (Novotny and Auffray, Nucleic Acids Res. (1984) 12:243-255) and recently confirmed by the crystal structure of the HLA-A2 molecule (Bjorkman et al., Nature (1987) 329:506-512; 512-518).

These CTL efficiently lysed syngeneic target cells in the presence of the H-2L^d derived peptide. Lysis of C3H cells by these CTL appeared to be dependent on the sequence and conformation of 61-85 H-2L^d peptide since no killing was observed in the presence of an 61-85 H-2D^d peptide or an analogue of the 61-85 H-2L^d peptide containing D-Gln and D-Leu residues. In addition, two unrelated peptides, a T-cell receptor peptide and a Class II peptide were unable to mediate lysis by these CTL. A population of anti-1591 CTL selected in parallel for growth on an H-2L^{149} L-cell transfectant instead of H-2L^d exhibited comparable anti-H-2L specific reactivity, lysing both the H-2L^d and A149 transfectants, but exhibiting no reactivity with the H-2L^d peptide. Therefore, selection for growth on H-2L^d appears to be important in order to enrich for the presumably small percentage of anti-H-2L CTL cells exhibiting peptide reactivity.

Recognition of the H-2L Peptide is MHC Restricted

In order to show that the H-2L^d peptide recognition is MHC restricted, a large panel of tumor cells and spleen cell blasts bearing various MHC haplotypes were examined for the ability to present this peptide to the H-2L reactive anti-1591 CTL. In the absence of H-2L^d peptide, H-2^d haplotype cells (BALB/c splenic blasts and P815 mastocytoma cells) were efficiently lysed, while no significant lysis was observed for the k, r, s, or f haplotype splenocytes. Of these four haplotypes, only H-2^k haplotype cells (C3H splenic blasts and L-cells) were lysed in the presence of the H-2L^d peptide. In addition, significant lysis of H-2^q

haplotype cells (BUB splenic blasts) was observed in absence of the peptide. This cross-reactivity app ars to reflect the strong structural homology which can be observed between two of the novel 1591 tumor antigens and H-2^q Class I molecules (Linsk, et al., supra; Lillehoj, et al., Proc. Natl. Acad. Sci. USA (1984) 81:2499-2503.

The antigen presentation activity of the hamster fibroblast line, tk^-ts13, and the human lymphoblastoid line, WIL 2C 13 were also tested. Neither of these targets were able to present the H-$2L^d$ peptide to the anti-1591 effectors. It is evident that the anti-1591 effectors recognize the H-$2L^d$ peptide in an H-2^k haplotype restricted manner. In addition, haplotype restriction of these CTL appears to be dependent on Class I MHC molecules, since L-cells are known to express no Class II products. Additionally, the anti-1591 CTL were unable to lyse the Class I negative, H-2^k haplotype thymoma, R1E in the presence of the H-$2L^d$ peptide.

In order to genetically map the H-2^k haplotype restriction element to a specific H-2^k Class I product, the ability of splenic blasts from two H-2^k derived recombinant inbred strains to present antigen to the peptide reactive CTL was examined. Neither H-2^r haplotype (RIIIS) nor H-2^s haplotype (A.SW) spleen cells appear to be capable of presenting the H-$2L^d$ peptide to the H-2L reactive anti-1591 CTL. Therefore, recombinant inbred mice derived from H-2^k and H-2^r or H-2^s haplotype progenitors were used to identify the H-$2L^d$ peptide restriction element. Neither B10.RKR(K^kD^r) nor B10.ASR2(K^kD^s) was able to present the H-$2L^d$ peptide to anti-1591 CTL, supporting that it is the H-$2D^k$ molecule which functions as the predominant restriction element for this population of peptide reactive CTL.

Anti-1591 CTL Recognize H-2L^d as an Independent Antigen

Since the anti-1591 CTL populations selected on the H-2L^d transfectant appeared to efficiently recognize the H-2L^d peptide in association with C3H L-cells, it was of interest to determine whether these CTL were also reactive with H-2L^d products in the absence of H-2^k haplotype restriction elements. To select a peptide reactive CTL population in the absence of the intact alloantigen, anti-1591 CTL was grown in the presence of untransfected L-cells with the H-2L^d peptide for eight weeks. These effectors were then shown to be able to lyse targets bearing intact H-2L^d. These cells also efficiently recognized H-2^d haplotype P815 as well as BALB/c blast target. Furthermore, lysis of BALB/c blasts was entirely dependent on the expression of an intact H-2L^d antigen, since BALB/c-H-2^{dm2}(K^dD^d) splenic blasts which lack the H-2L^d product were not lysed by the anti-peptide effectors and appeared to be unable to present the H-2L^d peptide. Therefore, the intact H-2L^d molecule functions either as a distinct antigenic target for the anti-H-2L^d effectors or alternatively functions as a restriction element presenting endogenous H-2L^d peptide to these CTL.

Peptide Activation of H-2L^d Specific CTL

The results described above suggest that the H-2L^d peptide in association with an H-2^k restriction element mimics an antigenic determinant exhibited by the intact H-2L^d molecule. To show that this antigenic homology reflects structural conservation which might be detected by other CTL reactive with the H-2L^d antigen, the ability of a panel of H-2L^d-reactive CTL clones to recognize the H-2L^d peptide was examined. Three of the clones tested, L3, L9.4, and L13.D.4 lysed C3H L-cells in the presence of the H-2L^d peptide. The fourth H-2L^d r active clone L13.D.8 appeard to exhibit

no reactivity with C3H L-cells plus the H-2L^d peptide, indicating that while this peptide appears to be an important antigenic determinant recognized by many H-2L reactive CTL, other H-2L reactive CTL appear to recognize distinct structures. These data are especially significant since two CTL, L3 and L9.4, were originally generated against target cells which do not express H-2^k haplotype Class I products (Linsk, et al, supra; Glasebrook and Fitch, supra (1979). Furthermore, as with the recognition of the H-2L^d peptide by the anti-1591 effectors examined above, peptide recognition by the CTL clone, L3, can be mediated by only H-2^k restriction elements.

Together, these results indicate the importance of amino acids 61-85 of the H-2L^d molecule as a recognition structure for many H-2L-reactive CTL. Furthermore, since the CTL which recognize this peptide were derived from a variety of H-2 backgrounds and yet were all able to utilize an H-2^k restriction element, H-2D^k appears to be uniquely suited to the presentation of this peptide.

The above data demonstrate that the H-2L^d peptide in association with H-2^k restriction element mimics a T-cell eptiope exhibited by the H-2L^d molecule. As evidenced by the crystallographic analysis of HLA-A2, the majority of the amino acid residues on the helices which are polymorphic among various Class I molecules are oriented toward the cleft. Nevertheless, the above data demonstrate that at least some of the polymorphic residues are directly recognized by the T-cell receptor, since a peptide derived from the α1 helix can reconstitute the antigenic structure seen by alloreactive T-cells on the intact molecule. The CTL studies not only recognized the peptide antigen in association with MHC, but also exhibited reactivity with allogeneic MHC products alone.

The above results demonstrate that peptides can be used to, in effect, change the nature of a restriction element of a host, so as to appear allogeneic to the CTL. Furthermore, by joining the binding domain or α helix of an allogeneic host with an antigen of interest, which may be cross-reactive with a pathogen, neoplastic cell or the like, a CTL response may be stimulated, so as to protect the host from subsequent exposure or to mount an attack against an infection or tumor. In this way, the host may by holistic effects be better able to respond to the disease and more rapidly recover from the disease.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A composition for use in a method for modulating the immune response of a cellular host to a first antigen employing a peptide comprising at least a binding domain, wherein said binding domain is characterized by comprising a discontinuous sequence of amino acids having homology with the amino acids associated with binding of one of the helices of a Class I antigen and at least one of:

(1) the interspersed amino acids defining an epitope having homology with a Class I immunodominant sequence of said first antigen;

(2) the interspersed amino acids being the same as the interspersed amino acids of said one helix, wherein said peptide is substantially free of the constant region of the Class I antigen naturally joined to said one helix; or

(3) either or both (1) and (2) joined to a Class I immunodominant sequence of said first antigen;

with the proviso that when said binding domain is (2), said binding domain may be bound to a Class I immunodominant sequence of a second antigen;

said method comprising:

administering to said cellular host an immune modulating amount of said peptide.

2. A composition for use in a method according to Claim 1, wherein said peptide is at least 10 amino acids and not more than about 60 amino acids.

3. A composition for use in a method for modulating the immune response of a cellular host to a first antigen employing a peptide comprising at least a binding domain, wherein said binding domain is characterized by comprising a discontinuous sequence of

amino acids having homology with the amino acids associated with binding of one of the helices of a Class I antigen of said host and the interspersed amino acids defining the epitope having homology with a Class I immunodominant sequence of said first antigen restricted by said host;

said method comprising:

administering to said cellular host an immune modulating amount of said peptide.

4. A composition for use in a method for modulating the immune response of a cellular host to a first antigen employing a peptide comprising at least a binding domain, wherein said binding domain is characterized by comprising a discontinuous sequence of amino acids having homology with the amino acids associated with binding of one of the helices of a Class I antigen of said host and the interspersed amino acids defining the epitope having homology with a Class I immunodominant sequence of said first antigen restricted by said host joined to a Class I immunodominant sequence of said first antigen or of a second antigen, where each of said Class I immunodominant sequences is restricted by a Class I antigen of said host;

said method comprising:

administering to said cellular host an immune modulating amount of said peptide.

5. A peptide of from about 14 to 60 amino acids comprising:

a discontinuous sequence of amino acids having homology with the amino acids associated with binding of one of the helices of a Class I antigen and at least one of:

(1) the interspersed amino acids defining the epitope having homology with a Class I

immunodominant sequence of said first antigen;

(2) the interspersed amino acids being the same as the interspersed amino acids of said one helix, wherein said peptide is substantially free of the constant region of the Class I antigen naturally joined to said one helix; or

(3) either or both (1) and (2) joined to a Class I immunodominant sequence of said first antigen;

with the proviso that when said binding domain is (2), said binding domain may be bound to a Class I immunodominant sequence of a second antigen.

6. A peptide according to Claim 5, wherein said Class I antigen is a murine Class I antigen.

7. A peptide according to Claim 5, wherein said binding domain comprises (1).

8. A peptide according to Claim 5, wherein said binding domain comprises (2).

9. A peptide according to Claim 5, wherein said binding domain is bound to a Class I immunodominant sequence of said first or second antigen.

10. A vaccine comprising a peptide according to Claim 5 in an amount sufficient to produce an immunogenic response in a physiologically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/00396

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 37/02; A61K 45/05; C07K 7/08; C07K 7/10.
U.S.: 514/12; 514/13; 514/14; 514/21; 530/324; 530/325; 530/326.

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/324, 530/325; 530/326, 530/402, 530/403, 530/406; 514/8, 514/12, 514/13, 514/14, 514/21; 424/85.8, 424/88.

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

On-line searches on BIOSIS, CAS, and APS.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	The Journal of Immunology, Vol.133, No.4, issued October 1984 (USA), Wabuke-Bunoti et al, "Cytolytic T lymphocyte and antibody responses to synthetic peptides of influenza virus hemagglutinin," pages 2194 to 2201.	1-10
Y	The Journal of Immunology, Vol.137, No.7, issued 1 October 1986 (USA), Singh et al, "Alloantigenic sites on class I major histocompatibility complex antigens: 61-69 region in the first domain of the H-2Kb molecule induces specific antibody and T cell responses," pages 2311 to 2318.	1-10
Y	The Journal of Experimental Medicine, Vol.162, No.2, issued 1 August 1985 (USA), Yamada et al, "Influenza virus hemagglutinin-specific cytotoxic T cell response induced by polypeptide produced in Escherichia coli," pages 663 to 674.	1-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 May 1989	07 JUN 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Jeff P. Kushan